Online Supplementary Material and Methods

Deletion of *Tmem43* Gene in Murine Cardiac Myocytes Activates TP53-TGFβ1 Pathways and Causes a Late-Onset Pro-fibrotic Cardiomyopathy

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Short title: Tmem43 haploinsufficiency causes pro-fibrotic cardiomyopathy

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Online Methods

***Myh6-Cre:Tmem43*$^{W/F}$ mice:** The *Tmem43* gene was deleted specifically in cardiac myocyte by crossing the *Myh6-Cre* deleter and *Tmem43*$^{tm1a(EUCOMM)Wtsi}$ mice, the latter generated by the Knock Out Mouse Project (KOMP). The KOMP mouse contains a LacZ reporter cassette cloned into intron 4 of the *Tmem43* gene flanked by flippase recognition target (FRT) sites as well as floxed exons 5 to 7 of the *Tmem43* gene (Online Figure 1). To excise the LacZ reporter cassette, the 129S4/SvJaeSor-Gt(ROSA)26Sor$^{tm1(FLP1)Dym/J}$ mouse expressing Flippase (JAX Stock No: 003946) was crossed to *Tmem43*$^{tm1a(EUCOMM)Wtsi}$ mice, which resulted in mice carrying one copy of the *Tmem43* gene containing the LoxP sites flanking exon 5-7 of the *Tmem43* gene. The *Tmem43* floxed (*Tmem43*$^{F/F}$) mice were crossed to *Myh6-Cre* mice to specifically delete exon 5-7 of the *Tmem43* gene in cardiac myocytes (*Myh6-Cre:Tmem43*$^{W/F}$).

Mouse Genotyping: The genotyping was performed by polymerase chain reaction (PCR) on genomic DNA extracted from mouse tails to specifically amplify a region spanning the LoxP site inserted in intron 7 of *Tmem43* gene. Genomic DNA was purified from mouse tail tissue using the Gentra Puregene mouse tail kit as described previously (Cat#158267, QIAGEN Inc, Valencia, CA). Mouse tail tissue (5-10 mg) was digested overnight at 55ºC in 300 µl of cell lysis solution containing proteinase K. Proteins were precipitated by mixing with 100 µl of protein precipitation solution followed by centrifugation for 5 min at 13,000g. The genomic DNA was precipitated with 100% isopropanol and washed with 70% ethanol. Finally, DNA pellets were resuspended in nuclease free water. A 100 ng aliquot of each DNA sample was used in the PCR reaction. The PCR cycles were as follows: 1 cycle for 5 min at 95ºC, followed by 35 cycles of denaturation at 95ºC for 15 sec, annealing at 58ºC for 30 sec, extension at 72ºC for 45 sec and the final extension cycle for 7 min at 72ºC. The sequence of the oligonucleotide primers used for genotyping is provided in Online Table1.

Recombination efficiency: Expand long template PCR system (Roche, Cat# 11681) was used to amplify a region spanning *Tmem43* gene from exon 4 to exon 8 (Online Figure 1) on DNA isolated from cardiac myocytes. The PCR conditions were; 2 min at 94ºC for initial denaturation followed by 10 cycles

at 94°C for 10 sec denaturation, 63°C for 30 sec annealing and elongation for 150 sec at 68°C. This stage was followed by 20 cycles including 15 sec at 94°C denaturation, 30 sec at 63°C annealing and 150 sec for elongation with gradually increase in extension time (+20 sec cycle elongation for each successive cycle) to obtain a higher yield of amplification products. Final elongation was at 68°C for 7min. Following DNA electrophoresis on 1% gel, the PCR products were visualized and the gel image was captured using Alpha Image 2200 system and the intensity of the PCR product bands were quantified by ImageJ software (https://imagej.nih.gov/ij/index.html). Recombination efficiency was calculated as a fraction of the flox allele.

Survival Analysis. Kaplan-Meier survival curves were plotted using GraphPad Prism 8 software (https://www.graphpad.com/) to compare the survival rate of the WT, *Myh6-Cre* and *Myh6-Cre:Tmem43*$^{W/F}$ mice over the course of about 20 months.

Gross morphology: Mice body weights were measured prior to euthanasia by administration of inhaled Co2 followed by cervical dislocation. Hearts were explanted immediately after euthanasia and washed in PBS at room temperature to remove excess blood. Heart weight was measured. Heart weight to body weight ratios were calculated for each mouse and the mean value per group of mice was compared among the groups.

Echocardiography: Echocardiography was performed in age and sex matched adult mice, as published. [1-3] The Vevo 1100 ultrasound imaging system (FUJIFILM VisualSonics Inc., Toronto, ON, Canada) equipped with a 22-55 MHz MicroScan transducer (MS550D) was used to obtain 2D and M mode images. The mice were positioned supine on a heating platform, the chest fur was removed with a hair removal cream and connected to ECG leads. Mice were kept under light anesthesia (0.5-1% isoflurane) to maintain the heart rate above 400 bpm during the procedure. The heart rate and cycle were continuously monitored. The measurement of indices including left ventricular and septal wall thicknesses as well as left ventricular internal dimensions during end systole and end diastole was performed by parasternal two-dimensional short-axis view at the tip of the mitral leaflet and recorded

from M-mode images. Each measurement was made for at least 5 cycles and the mean values were used. Left ventricular fractional shortening and mass were calculated from the measured indices.

Quantification of interstitial fibrosis: Hearts were rapidly excised from the chest cavity after euthanasia, washed in PBS, and fixed at 4ºC in 10% buffered formalin overnight. After fixation, the thick myocardial sections were dehydrated by soaking in a series of ethanol bath gradients ranging from 70 to 100%, washed in room temperature with xylene for 2 times, followed by incubation in 60 ºC paraffin for one hour, and then, were embedded in paraffin. The embedded paraffin thick sections were cut into 5 µm thin sections and preserved on superfrost® plus charged slides (VWR, cat# 48311-703). Further, the thin sections were deparaffinized in xylene, rehydrated in ethanol bath gradients from 100 to 50 %, and stained with picrosirius red (Sigma Aldrich, cat# P6744-1GA) or Masson trichrome (HT15-1KT, Sigma Aldrich, cat# 365548). An Olympus microscope equipped with a digital camera (Olympus BX40) was used to capture the images. Collagen-stained area in each myocardial section was quantified from picrosirius red stained sections and collagen volume fraction (CVF) was calculated as a percentage of the total myocardial area using ImageJ software (https://imagej.nih.gov/ij/index.html). At least 10-15 fields per section and 5-6 sections per heart and 5 mice per genotype were used to determine CVF. [1-4]

TUNEL assay: In-Situ Cell Death Detection Fluorescein Kit (Roche, cat # 11684795910) was used to perform terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay to detect apoptosis Briefly, the thin myocardial tissue sections, cut from paraffin-embedded heart tissues, were deparaffinized as described previously, and treated with proteinase K solution (10-20 Ug/ml in 10 mMTris/HCl, PH 7.4-8) for 20 min at 37°C. Further, the tissue sections were incubated with deoxynucleotidyl transferase dUTP nick end labeling solution (TUNEL mixture including label and enzyme solution) for one hour at 37 °C. Tissue sections were treated with DNase I recombinant (3000U/ml-3U/ml in 50 mM Tris –HCl, PH 7.5, 1 mg/ml BSA) for 15 min at room temperature and tissue sections treated only with the label solution (without terminal transferase enzyme) were used as positive and negative controls respectively. The sections were washed 3 times, 10 min per wash with PBS, incubated with DAPI for 10 min, washed 3time for 10 min each with PBS, mounted with DAKO

mounting media (cat# S3023) and visualized by Zeiss, Axioplan fluorescence imaging system. A total of 12,000 to 20,000 DAPI positive nuclei were counted and TUNEL positive cells were calculated as the percentage of total DAPI positive nuclei in each heart. [1-4]

Wheat Germ Agglutinin (WGA) staining: WGA staining was performed as described previously. [5] Thin myocardial sections from paraffin-embedded heart tissues were deparffinized, boiled in 10mM Sodium Citrate, pH 6 for 20 min and allowed to cool to room temperature. The sections were then permeabilized for 30 min with PBS containing 0.5% Triton X-100 followed by incubation with WGA conjugated to Texas red (Thermo Fisher Scientific, Cat#W21405) at a concentration of 1µg/ml for one hour. The sections were counter stained with DAPI (Sigma-Aldrich, St Louis, MO; cat# D8417) and visualized by Zeiss, Axioplan Fluorescence Microscope). Pericentriolar membrane protein (PCM1) antibody (Sigma, Cat# HPA023370), which specifically tags cardiac myocytes nuclei, was used to determine the number of cardiac myocytes in each section. (Bergmann, 2012 #1918;Bergmann, 2011 #651) Images were analyzed by Image J software (https://imagej.net), WGA stained pixel counts were subtracted from total pixel counts in each field and the residual pixels were divided by the number of c cells stained positive for PCM1, i.e., myocytes, to obtain average myocyte pixel count. At least 10-15 sections per mice and at least 5 mice per specific genotype was analyzed representing a total of 15,000 to 20,000 cells per heart.

Isolation of adult mouse cardiac myocytes: Adult cardiac myocytes were isolated as published previously with minor modifications. [1-4] Briefly, mice were anaesthetized using pentobarbital and excised hearts were placed in a perfusion buffer containing (120 mM NaCl, 15 mM KCl, 0.6 mM KH2PO4, 0.6 mM Na2HPO4, 1.2 mM MgSO4, 7H2O, 30 mM Taurine, 4.6 mM NaHCO3, 10 mM HEPES, 10 mM 2,3-Butanedione monoxime (BDM), and 5.5 mM Glucose; and pH was adjusted to 7.0). Hearts were cannulated retrogradely and perfused with a digestion buffer containing 2.4 mg/ml of Type 2 collagenase (Worthington Cat# LS004176) with adjusted flow rate of 4 ml/min at 42 °C for 3min followed by addition of 2mM CaCL2 and continued the enzymatic digestion for additional 5 minutes. Upon completion of the enzymatic digestion, vessels and atria were removed and ventricles were minced in a

stop buffer containing 10% calf serum, 12.5μM CaCl2, and 2 mM ATP. Using a 100 μm cell strainer the cell suspension was filtered and cardiac myocytes were pelleted at 20g for 4 min. Calcium reintroduction was performed in an increasing molar concentration of CaCL2 from 100 μM, 400 μM and 900 μM in the stop buffer. The cardiac myocytes were incubated for 4 min in each step of calcium reintroduction followed by centrifugation at 20 g for 4 min. After last CaCl2 reintroduction step, cardiac myocytes were suspended in a Qiazol reagent (Qiagen Cat# 79306) for subsequent RNA extraction or in protein extraction buffer to perform RNA-seq, RT-PCR, western blot and active TGFβ assay.

Immunofluorescence: Immunofluorescence staining was performed on isolated cardiac myocytes and myocardial tissue sections as described previously. [1-4] Hearts were explanted upon euthanasia, were placed in optimal cutting temperature (OCT) compound (Sakura Finetek Cat# 4583) and flash frozen in 2-Methyl butane precooled with liquid nitrogen. The OCT embedded hearts were used to cut 5μm thin myocardial sections. The sections were fixed in 4% formaldehyde solution at room temperature for 5 minutes and washed two times in PBS. The sections were blocked for at least an hour in blocking buffer containing 5% donkey serum in PBS containing 0.3-0.5% Triton X-100. Further, the sections were incubated with corresponding primary antibody in a blocking buffer containing 1% BSA overnight at 4°C to detect the expression and localization of proteins of interest including anti-PCM1 (Rabbit, Sigma, Cat# HPA023370) at 1:1000 dilution, anti-TMEM43 (Rabbit, Abcam, Cat# 184164) at.1:250 dilution, Anti-α-SMA (Mouse, Abcam, Cat# 18147-250) at 1 in 1000 dilution, anti-PDGFRα (Rabbit, Cell Signaling, Cat#3174S) at 1 in 1000 dilution, anti-pH2AFX at 1 to 200 dilution (EMD Millipore Cat# 05-636) . After three washes in PBS, sections were incubated in fluorescence labelled anti-mouse or anti Rabbit secondary antibody conjugated to Alexa Fluor 488 or Alexa Fluor 594 (Invitrogen, Cat# 21207, 21202) for one hour at room temperature. After three washes in PBS, sections were counter stained in 4′, 6-Diamidino-2-phenylindole di-hydrochloride (DAPI, Sigma, Cat# D8417) for 10 minutes, followed by three washes in 1X PBS and mounted in fluorescence mounting media (DAKO, Cat# S3023). Image acquisition was done using Zeiss, Axioplan fluorescence microscope.

Immunoblotting: Immunoblotting was performed as published. [1-3, 6-8] Total protein was isolated from mouse ventricular tissue or cardiac myocytes of wild type and *Myh6-cre:Tmem43*$^{W/F}$ mice. The ventricular tissue or isolated cardiac myocytes were homogenized and dissolved in a RIPA buffer (Cat# 974821) containing 0.5-1% SDS along with protease and phosphatase inhibitors (Roche, Cat#04693159001, 04906837001). The lysates were sonicated for 30 sec using Bioruptor Pico (Diagenode) and spun down at 13,000 rpm for 10 min at 4^0C. Around 50µg total protein was resolved on 4-20 % SDS PAGE gel and transferred onto nitrocellulose membrane. The membranes were probed for target proteins as indicated in the online table 1B including anti-TMEM43 (Abcam, Cat# 184164), pSMAD2, pSMAD3, SMAD2, and SMAD3 using the SMAD2/3 antibody sampler kit (Rabbit, Cell Signaling, Cat#12747). Likewise, the membranes were probed with an antibody against TGFβ1 (Mouse, R&D, Cat# MAB1835), CDKN1A (Mouse, Pharmingen, Cat# 556431), TP53 (Rabbit, Santa Cruz, Cat# 6243), p-P53 (Rabbit, Cell Signaling, Cat# 9281 and 9284), ATM (rabbit, Cell Signaling, Cat# 2873), pATM (Rabbit, R&D, Cat# AF1655), pH2AX (Mouse, Millipore, Cat#05-636), H2AX (Rabbit, Millipore, Cat# AB10022), LGALS3 (Rabbit, Abcam, Cat# 53082), VCAN (Rabbit, Abcam, Cat# 19345) and GDF15 (Rabbit, Abcam, Cat# 39999) . The membranes were stripped and probed for GAPDH as the endogenous loading control (Abcam, Cat# 8245). The ECL western blotting detection kit (Amersham Cat# RPN2106) and the LiCOR (Odyssey) imaging system were used for further signal detection.

RNA-sequencing: RNA-Sequencing was performed as described recently modifications. [1-3, 6] The miRNeasy Mini Kit from Qiagen (Cat # 217004) was used to isolate total RNA from isolated cardiac myocytes from mouse ventricular myocardial tissue. Integrity of the RNA extracts was confirmed by measuring RNA Integrity Number (RIN) on an Agilent Bioanalyzer RNA chip. Samples with RIN >8 were used to prepare sequencing libraries. TruSeq stranded total RNA library preparation kit that employ ribosomal RNA depletion (Illumina Inc. Cat # 20020596) was used to generate strand-specific sequencing libraries. Sequencing was performed on an Illumina HiSeq 4000 instrument for 75 bp, paired-end reads. RNA sequencing reads were aligned to the mouse reference genome build mm10 using HISAT2. (Kim, 2019 #2498) The aligned read pairs were annotated using GENCODE gene model

(https://www.gencodegenes.org/mouse/). Read counts were obtained using the featureCount and the differential expression analysis was performed using the DESeq2 statistical package in an R. [9-12] Significance level was set at a Benjamini-Hochberg FDR-adjusted p value of <0.05.

Pathway analysis: To obtain robust data on the pathways and gene set that are enriched from the RNA-seq data, we performed an *Ensemble of Gene Set Enrichment Analyses (EGSEA)*. (Alhamdoosh, 2017 #2500) *EGSEA* combines results from up to 12 prominent gene set testing algorithms to obtain a consensus ranking of biologically relevant results. For this analysis count matrix obtained from featureCount was used as input and the data was normalized and differential expression analysis was performed in EGSEA. The pathways enrichment was performed using Base GSEA methods namely camera (limma:3.38.3), safe (safe:3.22.0), gage (gage:2.32.0), globaltest, ora (stats:3.5.1) using Hallmark Signatures, GO Gene Sets and GeneSetDB Gene Regulation database. Significant pathways and Geneset were obtained based on combined q<0.05 and presented based on combined ranks.

Individual Gene set analysis was also performed using the GSEA for expressed genes in experimental samples (*Myh6-Cre:Tmeme43*$^{W/F}$ mice) and control samples (Wild Type mice). For GSEA, significance was assessed by analyzing signal-to-noise ratio and 1,000 cycles of gene permutations against the curated gene sets for hallmark and canonical pathways of Molecular signature database (MSigDB). Hypergeometric distribution function of GSEA was also used to obtain enriched gene set. The data were presented as enrichment score and an FDR cutoff of 0.05 to identify significant gene sets. When presenting results for specific gene sets nominal enrichment score and FDR values were shown.

Upstream regulators Analysis: The Upstream Regulator Analysis module of Ingenuity Pathway Analysis software (IPA®, QIAGEN Redwood City) was used to predict transcriptional regulators of differentially expressed genes (q value <0.05). A cutoff of Z score greater than +2 or less than -2 was used to identify significantly changed upstream regulators. Upstream regulators and the corresponding p-values and Z scores were plotted in R.

Quantification of active TGFβ1 level: TGFβ1 protein levels in isolated cardiac myocytes from wild type and *Myh6-Cre:Tmem43*$^{W/F}$ mice were determined using the Quantikine ELISA kit for Mouse/

Rat/ Porcine/Canine TGFβ1 from R&D Systems (Cat. # MB100B) as per the manufacturer's instructions. Briefly, cardiac myocytes were lysed in a buffer containing 50 mM Tris PH 7.4, 150mM Nacl, 1mM EDTA, 1mM EGTA, 0.5% Triton-X-100 and 0.1% Sodium deoxy cholate along with protease and phosphatase inhibitors. Assay was performed using 200µg of protein lysate after activating latent TGFβ1 by addition of 1N HCL followed by neutralization in 1.2N NaOH. Samples for standard curve was generated using TGFβ1 protein at dilutions ranging from 2000, 1000, 500, 250, 125, 62.5, 31.3 pg/ml. TGFβ1 standards and samples were loaded on to a pre-coated wells containing monoclonal antibody specific for TGFβ1 and incubated for 2hr at room temperature. Upon completion of the reaction, the unbound substances were washed away and an enzyme-linked polyclonal antibody specific for TGFβ1 was added to the wells and incubated at room temperature for 2 hours. The wells were then washed with a wash buffer for 3 times to remove the unbound material according to the manufacturer protocol. Colorimetric detection of TGFβ1 was performed following addition of a substrate solution containing hydrogen peroxide and tetramethylbenzidine. Microplate reader (https://lifesciences.tecan.com) was used to measure the reaction color intensity. TGFβ1 level was calculated and plotted as pg/mg protein.

PP2A activity assay: PP2A Immunoprecipitation Phosphatase Assay Kit (millipore, Cat# 17-313) was used to measure PP2A activity in cardiac myocytes lysate. Briefly, isolated cardiac myocytes were lysed in a lysis buffer containing 20 mM Imidazole-HCl, 2mM EDTA, 2mM EGTA, PH 7, with protease inhibitor. The lysates were incubated with 4 µg of PP2A antibody (C subunit, clone1D6, Cat#05-421) together with agarose beads overnight with constant shaking. The beads were washed with 700 µl TBS, followed by one wash with 500 µl Ser/Thr assay buffer. The premade diluted phosphopeptide (1 mg threonine phosphopeptide, Cat# 12-219, dissolved in 1.10 ml of distilled water for a final concentration of 1 mM) was added together with Ser/Thr assay buffer, followed by 10 minutes incubation at 30°C on a shaker and a brief centrifugation. Further, the samples along with controls and standard samples were transferred into each well of a microplate reader, incubated for 15 minutes at room temperature upon addition of Malachite Green phosphate detection solution according to the manufacturer protocol. The

microplate reader (https://lifesciences.tecan.com) was used to measure the absorbance values of the samples representing PP2A activity level and plotted as picomole of phosphate per mg of protein.

Co-immunoprecipitation (Co-IP): Co-IP was performed as published with minor modifications. [13] In brief, adult mouse whole heart tissues were washed in cold PBS, minced into small pieces and homogenized using a hand held homogenizer. The tissue was incubated in a buffer containing 50mM Tris pH 7.4, 150mM NaCL, and 0.5% Triton-X-100 along with protease and phosphatase inhibitors (Roche Cat# 11 836 153 001, Cat# 04 906 845 001) for 2hr in a shaker at 4ºC. The tissue lysates were disrupted by sonication using Bioruptor Pico (Diagenode) for 30 sec at 4ºC. Lysates were spun down by centrifugation at 13,000 rpm for 15 min at 4^0C. The supernatants were used to measure protein concentration. Approximately, 500µg of protein lysate was precleared with protein G sepharose for 1hr at 4^0C and incubated with either an TMEM43 (Abcam Cat# 184164) or TGFβ1 antibody (R&D systems Cat#MAB1835) overnight at 4^0C. The protein immune complexes were pulled down by BSA pre-coated protein G Sepharose beads for 1hr at 4^0C. The complexes were washed at least 5 times with 50mM Tris pH 7.4, 150mM NaCL and 0.5% Triton-X-100 and 2X Laemmli sample buffer was added (Bio-Rad Cat#161-0737). The samples were then heated at 95^0C for 5 min, resolved on SDS-PAGE gel, transferred to a membrane, and the membrane were then probed with either an TMEM43 or TGFβ1 antibody overnight at 4^0C. The signal was detected using HRP conjugated secondary antibodies (Cell signaling Cat#7076) and ECL western blotting detection kit (Amersham Cat# RPN2106) in a LiCOR (Odyssey) imager.

Chromatin immunoprecipitation: Chromatin immunoprecipitation was performed to determine interactions of TMEM43 and lamin A/C (LMNA), as described previously. [6] Briefly, total heart tissue was crosslinked in 1% formaldehyde (Sigma, Catalog no. F8775) for 10 min at room temperature. The reaction was stopped by adding 125mM glycine followed by three washes with cold PBS at 800g for 5 min. Nuclei were isolated from the tissue by homogenizing in a Dounce homogenizer using a type A pestle for 8 strokes in a buffer containing 20mM Tris pH 8.0, 2mM EDTA 0.1% Triton-X-100 and 10%

glycerol along with protease and phosphatase inhibitors (Roche Cat# 11 836 153 001, Cat# 04 906 845 001). The tissue homogenate was filtered through 100 µm filter (VWR Cat#732-2759) and nuclei were pelleted at 800g for 8 min at 4^0C. Nuclei were lysed in a buffer containing 10mM Tris pH 8.0, 140mM NaCl, 1mM EDTA, 0.5mM EGTA, 1% SDS, 0.1% Sodium deoxy cholate and 1%Triton X-100 along with protease and phosphate inhibitors. The lysates were sonicated in a Bioruptor Pico (Diagenode) for 15 cycles with 30sec On/Off at 4^0C to achieve a fragment size of 200-500bp. The sonicated lysates were diluted by 10 times and precleared with 30µlt of Protein G Sepharose (GE Healthcare Cat# 17-0618-01) for one hour at 4^0C. Precleared lysates were incubated with 5µg of TMEM43 antibody (Rabbit, Abcam, Cat# 184164) overnight at 4^0C along with control rabbit IgG (Cat#12-370). The immune complexes were pulled down with precoated BSA protein G Sepharose beads for one hour at 4^0C. The pulled down complexes were washed two times with a low salt wash buffer (20 mM Tris pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% TritonX-100, 0.1% SDS), then a high salt wash buffer (20 mM Tris pH 8.0, 500 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS), then, a LiCl wash buffer (20 mM Tris pH 8.0, 500 mM LiCl, 2mM EDTA, 1% TritonX-100, 0.1%SDS) followed by a TE wash buffer (10mM Tris pH 8.0, 1mM EDTA pH8.0). The beads were resuspended in a 2XLaemmli sample buffer (Bio-Rad Cat#161-0737), heated at 95 ºC for 10 min followed by resolving protein lysates onto an SDS-polyacrylamide gel electrophoresis system) and transferred to a nitrocellulose membrane. The membrane was blocked in 5% BSA in 1XTBST followed by incubation with an anti-LMNA antibody (Cat#ab26300) over night at 4^0C. The membrane was incubated with HRP conjugated secondary antibody (Cell signaling Cat#7076) followed by detection using the ECL western blotting detection kit (Amersham Cat# RPN2106) with a LiCOR (Odyssey) imaging system. Same membrane was stripped and probed for anti-TMEM43 antibody.

Statistical analysis: Normally distributed data, presented as mean ± SD, were compared by t-test or one-way ANOVA, followed by pairwise Bonferroni's multiple comparison test. Data deviating from a Gaussian distribution were presented as the median values and analyzed by Kruskal-Wallis test using Graph pad Prism 8 (www.graphpad.com/scientific-software/prism/).

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Table 1- Online

Oligonucleotide Primers, Antibodies, and TaqMan probes

A. Primers used for genotyping

Transgene	Sequence
Myh6-Cre	Forward: ATGACAGACAGATCCCTCCTATCTCC Reverse: GCGAACCTCATCACTCGTTGCATCGA
TMEM43$^{F/F}$	Forward: AGTCCTCAGGGGGTAGAACC Reverse: GAACTCCTGGCTGAGAGCTG

B. Antibodies

Antibodies	Concentration	Supplier	Catalog number
Anti-mouse IgG HRP linked antibody	1:4000 (IB)	Cell Signaling Technology	7076
Anti-rabbit IgG HRP linked antibody	1:2000 (IB)	Cell Signaling Technology	7074
Anti-goat IgG HRP linked antibody	1:1000 (IB)	Santa Cruz	2354
Donkey anti-Rat IgG, Alexa Fluor 488	1:1000 (IF)	Invitrogen	A21208
Donkey anti-Mouse IgG, Alexa Fluor 488	1:1000 (IF)	Invitrogen	A21202
Donkey anti-Rabbit IgG, Alexa Fluor 594	1:1000 (IF)	Invitrogen	A21207
GAPDH	1:1000 (IB)	Abcam	8245
TMEM43	1:1000 (IB), 1:50 to 1:200 (IF)	Abcam	184164
TMEM43	1:50 (IF)	Santa Cruz	271887
α-SMA	1:1000 (IF)	Abcam	18147-250
PDGFRα	1:2000 (IF)	Cell Signaling	3174S
SMAD2	1:1000 (IB), 1:100 (IF)	Cell Signaling Technology	12747
SMAD3	1:1000 (IB), 1:100 (IF)	Cell Signaling Technology	12747
pSMAD2	1:500 (IB), 1:10 (IF)	Cell Signaling Technology	12747
pSMAD3	1:1000 (IB), 1:100 (IF)	Cell Signaling Technology	12747
TP53	1:250 (IB)	Santa Cruz	6243
p-P53(S15, mouse S18)	1:1000 (IB)	Cell Signaling Technology	9284
p-P53(S392, mouse 389)	1:1000 (IB)	Cell Signaling Technology	9281
CDKN1A	1:500 (IB)	Pharmingen	556431
TGFB1	1:1000 (IB), 1:50 (IHC)	R&D	1835

PLIN1	1:100 (IF)	Cell Signaling Technology	9349
CEBPA	1:1000 (IF)	Cell Signaling Technology	8178
TMEM173(STING1)	1:1000 (IB)	Abcam	92605
CGAS	1:1000 (IB)	Cell Signaling Technology	15102
GALECTIN3 (LGALS3)	1:1000 (IB)	Abcam	53082
VERSICAN (VCAN)	1:1000 (IB)	Abcam	19345
GDF15	1:1000 (IB)	Abcam	39999
ATM	1:1000 (IB)	Cell Signaling Technology	2873
pATM(S1981)	1:500 (IB)	R&D	AF1655
pH2AFX	1:1000 (IB), 1:200 (IF)	EMD Millipore	05-636
H2AX	1:1000 (IB)	Millipore Sigma	AB10022
Caspase-3(D3R6Y)	1:1000 (IB)	Cell Signaling Technology	14220
Cleaved Caspase3(Asp175)(5A1E)	1:1000 (IB)	Cell Signaling Technology	9664

C. Oligonucleotide primers used in qPCR reactions

Name	**Sequence**
Gapdh	Forward: AACTTTGGCATTGTGGAAGG Reverse: GGATGCAGGGATGATGTTCT
Tgfb1	Forward:TGGAGCAACATGTGGAACTC Reverse: GTCAGCAGCCGGTTACCA
Tgfb2	Forward: AGGAGTGGCTTCACCACAAAGACA Reverse:ATTAGACGGCACGAAGGTACAGCA
Timp1	Forward: CATGGAAAGCCTCTGTGGATA Reverse: CTCAGAGTACGCCAGGGAAC
Dgat1	Forward: GAGGTGCGAGACGCGG Reverse: ATGGCACCTCAGATCCCAGTA
Bnip3	Forward: TCCTGGGTAGAACTGCACTTC Reverse: GCTGGGCATCCAACAGTATTT
Gadd45g	Forward: GGGAAAGCACTGCACGAACT Reverse: AGCACGCAAAAGGTCACATTG
Gadd45b	Forward: CAACGCGGTTCAGAAGATGC Reverse: GGTCCACATTCATCAGTTTGGC
Bcl2l11	Forward: CCCGGAGATACGGATTGCAC Reverse: GCCTCGCGGTAATCATTTGC
Ogdhl	Forward: GCCAGCTCTACGACTGCAAC Reverse: GTGAAGACAATGAGAGGCTTGCG
Klhdc7a	Forward: ACCAGGCAAGTGATTCCAGG Reverse: TCAGCGCCCCTTCTTTCAAT
Kcnj14	Forward: TCAGTGAGAACGCAGTGGTG

	Reverse: GGTCACACGGGGCTGC
Ucp2	Forward: AATGGGGGAGACGAGGAAGA Reverse: CTGCGGTCCGGACACAATAG
Ephx1	Forward: GCCACTGCGAGGATCTTCTA Reverse: GGGAATAAATGTTTTTGGGCACC
Bcat2	Forward: CCCACTGTGGCTGTGC Reverse: TCCAGTACTCCGTCTTCATGG
Cgnl1	Forward: TCACCCCGAAAGCCCTTAAA Reverse: GGTTCTCTGTCCCAGCTTGA
Acta2	Forward: CCAGCCATCTTTCATTGGGATG Reverse: TACCCCCTGACAGGACGTTG
Scn1b	Forward: AAGATTGCTGCTGCTACGGA Reverse: CCAGAGCCAGCGCTATTCAG
Bcl2	Forward: CACCCCTGGTGGACAACATC Reverse: GTTCCACAAAGGCATCCCAGC
Pcolce	Forward: GGGCACTGAGCACCAGTTTT Reverse: GCTGATGCCTGGTGGGTAAT
Uchl1	Forward: CGGCCCAGCATGAAAACTTC Reverse: CGTGGATCAACCCGATGGTA
Dmd	Forward: CTCACTGCCTGTGAAACCCT Reverse: TCAGGTCAGCTAAAGACTGGT
Ctnna3	Forward: TGGTGCTCAGCTAAAGGGAC Reverse: CCGACATGTTGCCTTTCTGAT
Lmod2	Forward: TAAGACAGCTACGGAGGGTGG Reverse: GGCATTTTGGGAACAGCTCAC
Abca12	Forward: TGTTTTGGCCTCCTTGGTGT Reverse: CCAGGGATCCGCTCTTGTTT
Ndnf	Forward: TCTTGGTAGGATCCCCGGAC Reverse: ACCAGTAGAACAGCTCCATCCTTA
Tbc1d17	Forward: CCAGCTACAGGGTGGTGTTT Reverse: TTCCACCACTCGTATGACGC
Slc9a3	Forward: AGTGATGGGAGCTTCCAGGT Reverse: ACAGGTGAAAGACGATTTTGGC
Cgnl1	Forward: TCACCCCGAAAGCCCTTAAA Reverse: GGTTCTCTGTCCCAGCTTGA
Ndrg4	Forward: GTCTACGTGTGTCGTGTTTCC Reverse: TCCCCCAACCAAATGTGTCT
Jpt	Forward: GGAACAGCTCCCGGGTTTT Reverse: GTTCTCTTCCGGTGTCCCAA
Guk1	Forward: ACATCAAGTAGGCATGGCAGG Reverse: AGTATGAGACACGCTGAAGCC
Fgf11	Forward: GATTCCTGTGGGACTCCGTG Reverse: CAGAACGACGCGAGCTGTA
Tmprss	Forward: TCATCCTTCTCTTCTACTTCTGGAG Reverse: CCCAGCTCATCGCTCTTCAT
Ano10	Forward: GCCGTAGAGCATGCACTCTT Reverse: GCCGTAGAGCATGCACTCTT
Otud1	Forward: CATCTTTCTGGGTAGAGTGGTTT Reverse: CTCTCAGGATTCTGGGACATATT
Kcnj3	Forward: CGAGCATGCGGTTATCTCCA

	Reverse: TGACAAGTCATTCTTTGAGCAGC
Kcnv2	Forward: GGGCCGCGGTAAGCATC Reverse: GTTGTAGAGGATGGAAATGGGC
Hcn1	Forward: GAAGGAGCTGTGGGGAAGAAA Reverse: GCAGGCATATCTCTCCGAAGT
Lmod2	Forward: TAAGACAGCTACGGAGGGTGG Reverse: GGCATTTTGGGAACAGCTCAC
Myot	Forward: ACACTAAGCTCAGAGCCACG Reverse: ATTGTTTCCTTAGCTCAGAGGGG
Clcn1	Forward: CCACAATGTGACAGACCTGC Reverse: CGCCAGTAATTCCGAACAGC
Prlr	Forward: GCTAGAGACTGGCTCTCCAAG Reverse: TGAGTCTGCTGCTTCAGTAGT
Aldob	Forward: TCCACGAGACCCTCTACCAG Reverse: CACCTCCTTGGTCCAACTTGA
Lama2	Forward: CCCAGGTTAAGGACCTGCAT Reverse: GTGCCTGCATCTGCAATGAT
Alox5	Forward: ATTGCCATCCAGCTCAACCA Reverse: ACTGGAACGCACCCAGATTT
Ppp2r3a	Forward: TCATTTCAGCAGACACAGATTCA Reverse: ACTCACACAAGGAACAGGGG
Cnot6l	Forward: TAGAGATCTCGGAGTGCGGA Reverse: ATCAGGTGGAATGCGAGCAA
Erbb4	Forward: ATGGCCTACAGGGAGCAAAC Reverse: TGGGACCGTTACACCCCT
Klhdc7a	Forward: ACCAGGCAAGTGATTCCAGG Reverse: TCAGCGCCCCTTCTTTCAAT
Klk1b22	Forward: TGTGTCCATCAAGCTCCATCC Reverse: GCCTCCTGAGTCTCCCTTAC
Vcan	Forward: GCAAGAAGGGAACAGTTGCT Reverse: TGGAAGGTGTCGCTGAATGA
Mis18bp1	Forward: AGAGGTAAGGGGCCAGAGTT Reverse: TGGCTTGTAATTTCAGGCTGC
Col5a2	Forward: GTACCACGGGCAAAGAGGAA Reverse: CTTTTCCTGGTGTACCCGCT
E2f1	Forward: GCATTGACTGACCTGCCTTGC Reverse: AAAGCCTGAGCGTGCACTAA
Fstl3	Forward: GATGGGCTCCGAGGATTCTGTG Reverse: TGGACCAGGCGGTGTTGAT
Phlda3	Forward: GACATGTCAAGCTTCTCTGTCC Reverse: GCTGGTTGGCTCCTTCCAT
POSTN	Forward: AGAGAAATCCCTCACGACA Reverse: GTTGGTGCAAACAAGGTCCA
Gdf15	Forward:CTCAACGCCGACGAGCTAC Reverse: ACCCCAATCTCACCTCTGGA
Ddias	Forward: TTGCCTCAGTACTTGCTGTCC Reverse: TGGGACAAGTAAACCTTTTGGA
Inhba	Forward: AGGTGCTGCTCAAGTGCCAAT Reverse: TTGTCAGCCGGCTCTTGTCT
Col6a1	Forward: CCTGGGGATCTTGGACCAGT

	Reverse:TTGCCTTTCTCGCCCTTGTA
Lgals4	Forward: CCGGGAGCCATGACTATTCC Reverse: CATATGGCACACGCGGATTG
Hadha	Forward: CAAGGGCTTCTTAGGTCGCA Reverse: GCAGCCTCAGATTTGCCAAG
Serpinb6b	Forward: CTGCAGCTATTTGAGGCCAGA Reverse: TGGATCCATGATGGTAAGCCTGG
Klc1	Forward: GCGTAGAGTGGAATGGGATGA Reverse: CTAGGCCATGGGACGCTG
Nol3	Forward: GGGACTATCCGAAACGCTC Reverse: GCACGTTGCCCATTTCTTCG
Stag1	Forward: AGTGCAATGCAGGTACGGTC Reverse: GGACCAGGCATTGTAAGGGG
Tollip	Forward: CAAGAGGTTGTGCTCCCCAG Reverse: GGAGTTCACCAATGTACACCTCA
Nfia	Forward: CCAGAACTTGGTGGATGGATGA Reverse: CCTGCAGGTTGAACCATGTG
Trdmt1	Forward: GAGAGGATGGAACCTCTGC Reverse: GCAGGGATATGACTTTCTCGC
Pdlim4	Forward: CACCATCTCGCGGGTTCAT Reverse: TTGGATCAAGTCACCTGGGC
Ccnb1	Forward: AAATTGCAGCTGGGGCTTTC Reverse: TGCAGAGTTGGTGTCCATTCA
Nox4	Forward: CCAAATGTTGGGCGATTGTGT Reverse: CAGGACTGTCCGGCACATAG
Nrg1	Forward: AGGACCTGTCAAACCCCTCA Reverse: AGAGCTCCTCCGCTTCCATA
Lgals3	Forward: TAATCAGGTGAGCGGCACAG Reverse: GCTAAGGCATCGTTAAGCGAAA
Gdf15	Forward: AACCCCTGGTCTGGGGATAC Reverse: CATGTCGCTTGTGTCCTTTCAG
Adamts8	Forward: TTGTTCACCAGACAGGGCAC Reverse: CACGTGGATGAAGAATGGCG
Serpinb1c	Forward: ACCTGCTAAGCAAGAGCCTTC Reverse: TGTGGAACAGCTCCAAAGCA
Sprr1a	Forward: TGAGTATTAGGACCAAGTGCTATCT Reverse: GTTTTGGGGGCACAAGGTTC
Cdkn1a	Forward: GGAACATCTCAGGGCCGAAA Reverse: TGGGCACTTCAGGGTTTTCT
Bax	Forward: ACAGGGGCCTTTTTGCTACA Reverse: CACTCGCTCAGCTTCTTGGT
Noxa	Forward: ATAACTGTGGTTCTGGCGCA Reverse: CAATCCTCCGGAGTTGAGCA
Puma	Forward: GAGACAAGAAGAGCAGCATCG Reverse: TAGTTGGGCTCCATTTCTGG
Caspe12	Forward:TTTCGAAAGGTTCAACACTC Reverse: CGTGTCATGGATACTCTCTC
Efnb3	Forward: ATCAGATACGGTTACCAGAG Reverse: CGTCTGATGTGGCAATTATG
Hspa12a	Forward: ATCAGATACGGTTACCAGAG

	Reverse: AAGGCAATCATTAGGTCAAC

D. TaqMan probes

Gene	TaqMan Assay ID
Gapdh	Mm99999915_g1
Col1a1	Mm00801666_g1
Col3a1	Mm00802300_m1
Adipoq	Mm00456425_m1
Apoe	Mm01307193_g1
Pparg	Mm00440940_m1
Cebpa	Mm00514283_s1
Dgat1	Mm00515643_m1
Myh6	Mm00440359_m1
Myh7	Mm0060555_m1
Nppa	Mm01255747_g1
Nppb	Mm01255770_g1
Acta2	Mm00725412_s1
Atp2a2	Mm01201431_m1
Ctgf	Mm01192932-g1

Online Table 2

Echocardiographic phenotype at ~ 4 months of age without cardiac dysfunction used for RNA-Seq

	WT	*Myh6-Cre:Tmem43* $^{W/F}$	*p*
N (m/f)	5 (3/2)	5 (3/2)	
Age (m)	4.1 ± 0.20	4.4 ± 0.30	0.1073
BW (g)	25.2 ± 2.16	26.6 ± 3.11	0.4642
HR (bpm)	496.4 ± 59.62	499 ± 45.25	0.9463
ST (mm)	0.6 ± 0.05	0.6 ± 0.04	0.2126
LVEDD (mm)	3.5 ± 0.28	3.3 ± 0.26	0.2693
LVEDDi (mm/g)	0.12 ± 0.02	0.15 ± 0.04	0.2552
PW (mm)	0.7 ± 0.06	0.8 ± 0.05	0.1083
LVESD (mm)	2.1 ± 0.22	2.3 ± 0.29	0.2480
FS (%)	34.5 ± 5.46	36.7 ± 2.32	0.4808
EF (%)	64.2 ± 6.83	67.7 ± 3.19	0.3806
LVM (mg)	60.4 ± 14.38	55.2 ± 10.39	0.5694
LVMi (mg/g)	2.3 ± 0.60	2.1 ± 0.54	0.4735

Abbreviations: *Myh6-Cre:Tmem43*$^{W/F}$: Cardiac myocyte specific heterozygous deletion of *Tmem43*; M/F: Male/Female; BW: Body weight; g: Grams; HR: Heart rate; bpm: Beats per minute; IVST: Interventricular septum thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVEDDi: LVEDD indexed to the body weight; LVESD: Left ventricular end systolic diameter; FS: Fractional shortening; LVM: Left ventricular mass; LVMI: LVM indexed to the body weight.

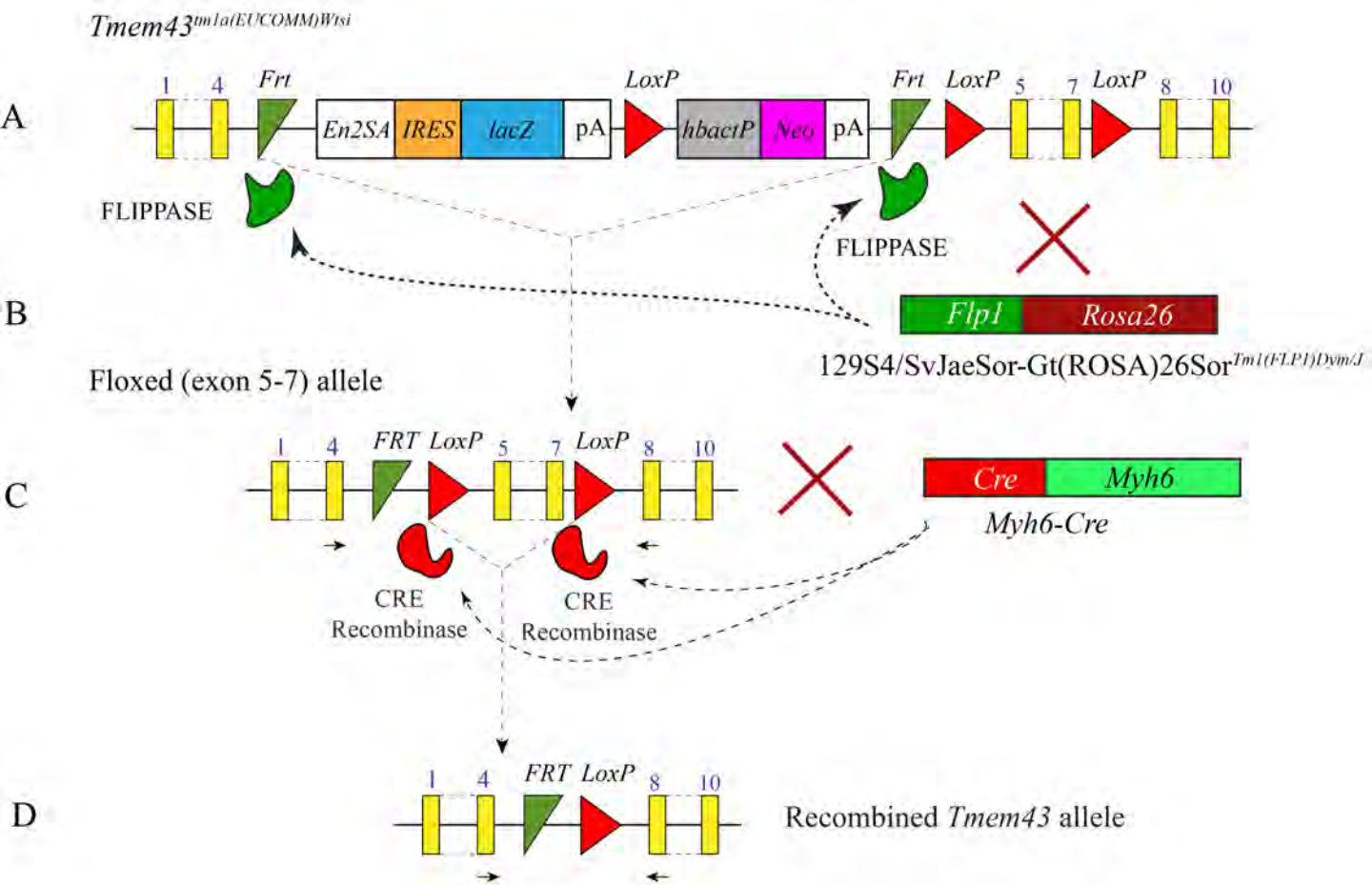
Online Table 3

Echocardiographic phenotype in ~ 9 months 0ld mice showing cardiac dysfunction used for RNA-Seq

	WT	*Myh6-Cre:Tmem43* $^{W/F}$	*p*
N (m/f)	6 (3/3)	6 (3/3)	
Age (m)	10.1±0.71	8.9±0.93*	0.0484
BW (g)	31.7±6.84	30.5±7.05	0.7819
HR (bpm)	483.1±24.50	501.3±28.35	0.3038
ST (mm)	0.69±0.07	0.7±0.10	0.9424
LVEDD (mm)	3.5±0.28	4.4±0.51*	0.0066
LVEDDi (mm/g)	0.11±0.03	0.15±0.03	0.0770
PW (mm)	0.85±0.07	0.8±0.05*	0.0173
LVESD (mm)	2.28±0.24	3.7±0.59*	0.0006
FS (%)	35.7±3.53	17.0±5.22*	0.0001
EF (%)	66.11±4.63	35.5±9.26*	0.0001
LVM (mg)	74.8±7.08	100.2±22.12*	0.0365
LVMi (mg/g)	2.4±0.61	3.4±0.70	0.0546

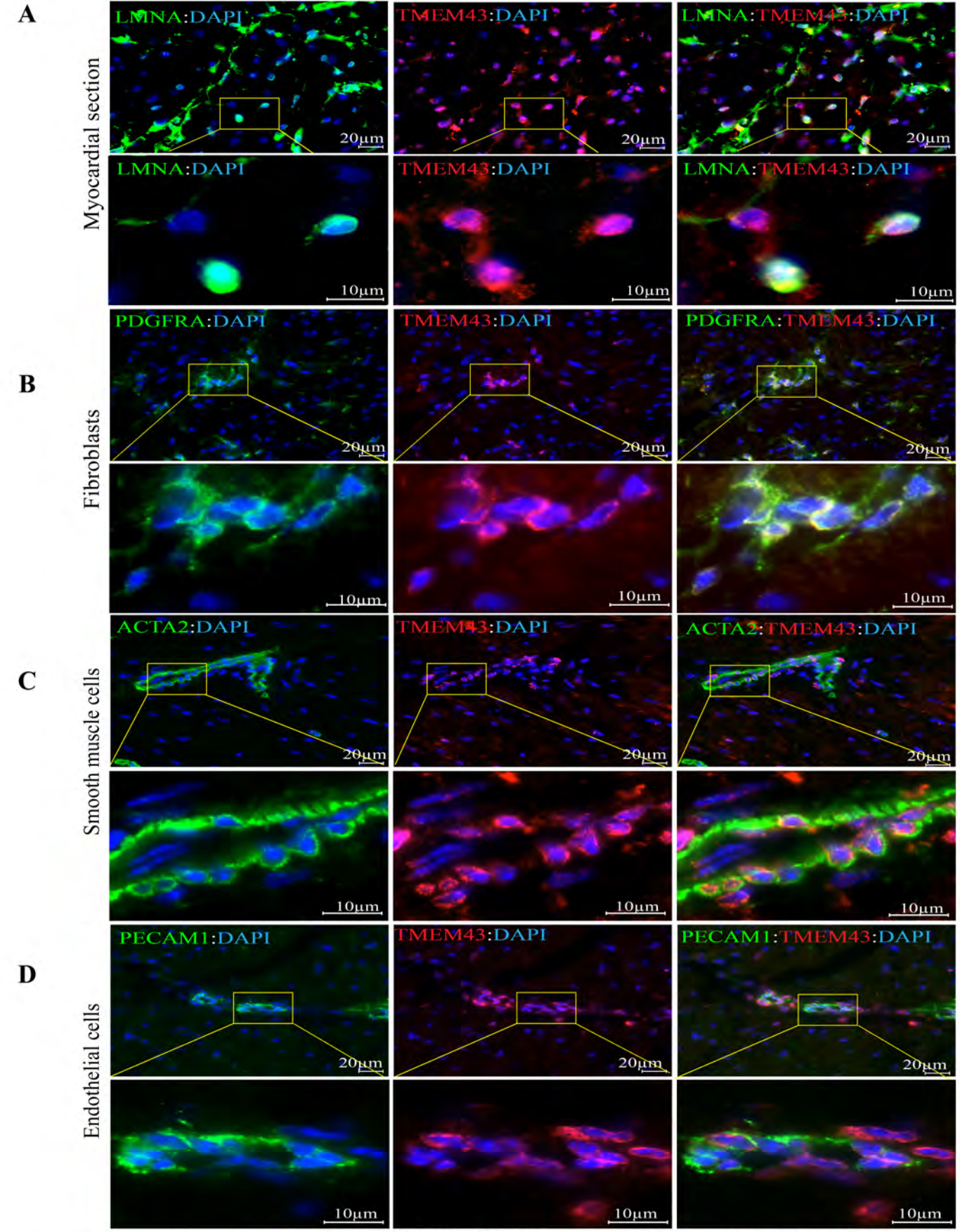
Abbreviations: *Myh6-Cre:Tmem43*$^{W/F}$: Cardiac myocyte specific heterozygous deletion of *Tmem43*; M/F: Male/Female; BW: Body weight; g: Grams; HR: Heart rate; bpm: Beats per minute; IVST: Interventricular septum thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVEDDi: LVEDD indexed to the body weight; LVESD: Left ventricular end systolic diameter; FS: Fractional shortening; LVM: Left ventricular mass; LVMI: LVM indexed to the body weight.

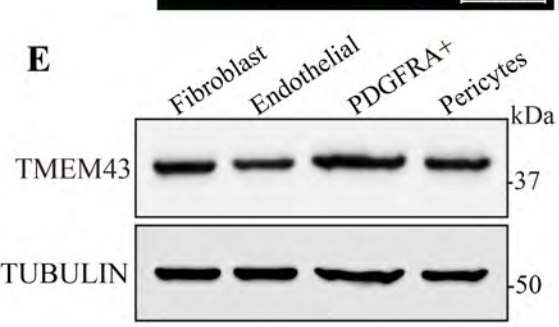
* denotes $p<0.05$



Online Figure 1. Generation of the *Myh6-Cre:Tmem43*$^{W/F}$ mice

A. Schematic representation of the Tmem43$^{tm1a(EUCOMM)Wtsi}$ allele containing Frt and LoxP sites along with the LacZ reporter cassette is shown.
B. Cartoon illustrating the Flippase mice, which excises the reporter cassette flanked by Frt sequences is depicted.
C. Crossing the *Tmem43* floxed (*Tmem43*$^{F/F}$) and $^{Myh6\text{-}Cre}$ mice deletes exon 5-7 of the *Tmem43* gene in cardiac myocytes (*Myh6-Cre:Tmem43*$^{W/F}$).
D. Schematic presentation of an excised allele upon recombination. Arrows indicated location of PCR primers used for detection of WT and recombined allele.





Online Figure 2. Detection of expression of TMEM43 protein in various cell types in the heart of wild type mouse.

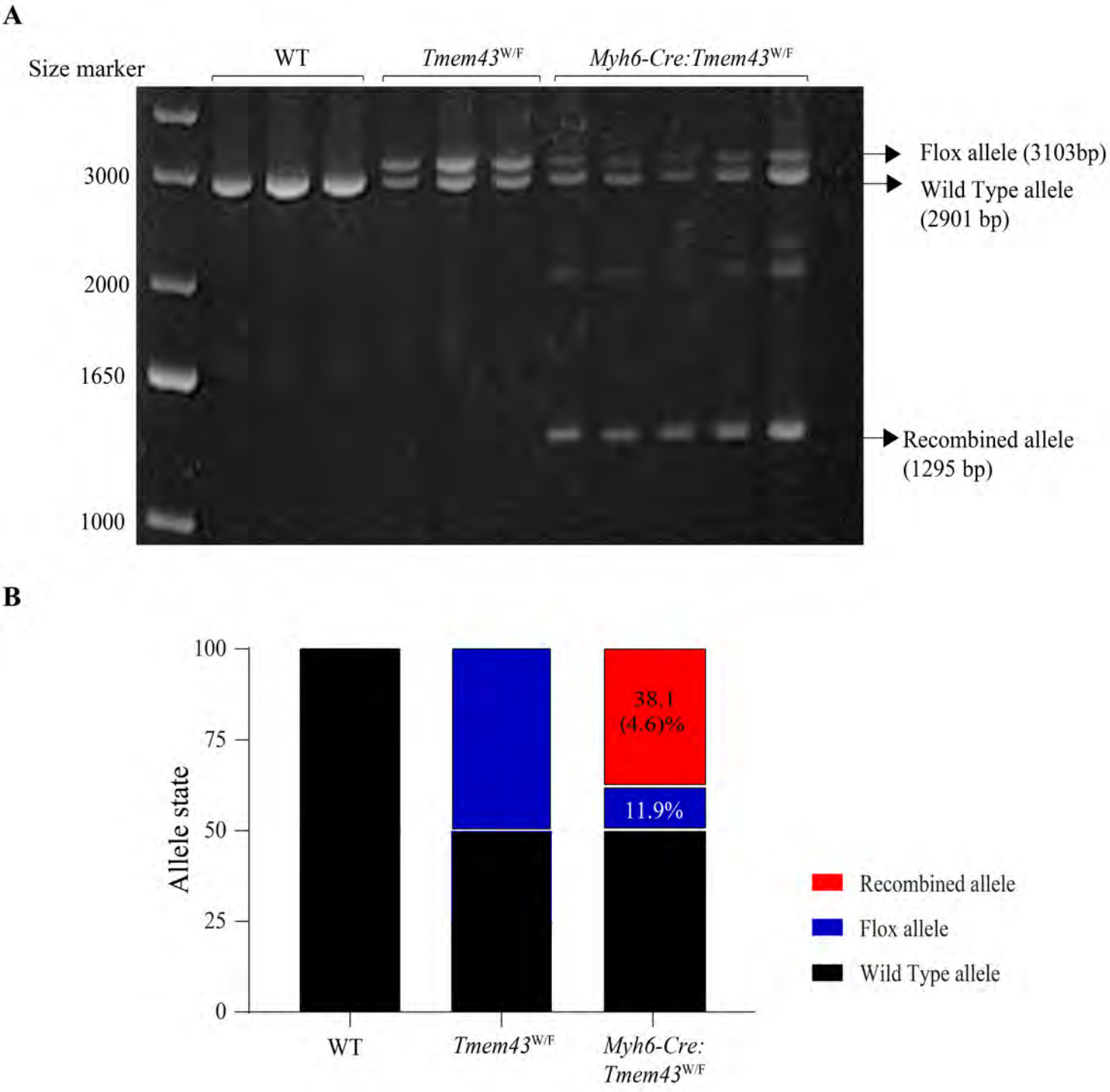
A. Low (upper) and high (lower) magnification immunofluorescent (IF) panels showing co-staining of myocardial sections with antibodies against LMNA and TMEM43 proteins

B. As in A, except that panels are stained with antibodies against TMEM43 and PDGFRA, the latter a marker of fibroblasts.

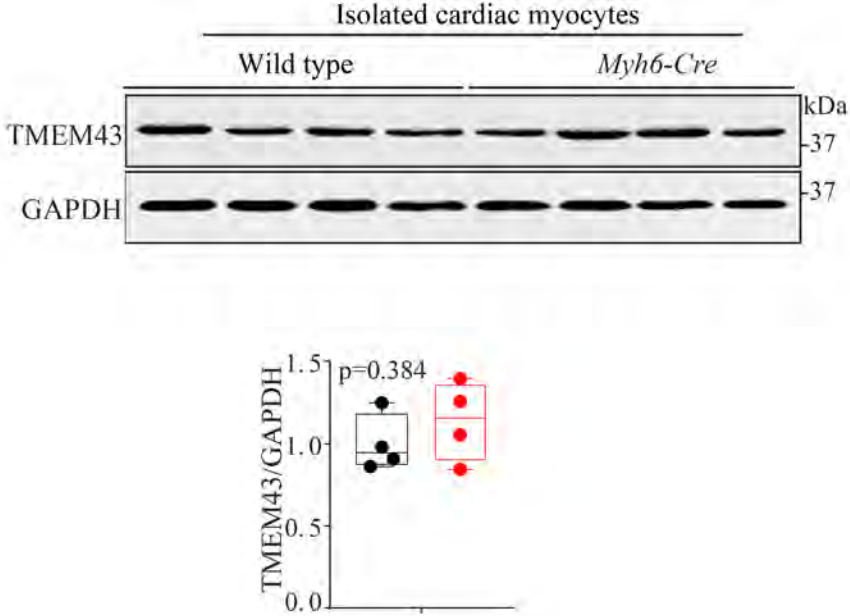
C. As in A, except for co-staining with antibodies against TMEM43 and α-smooth muscle actin (ACTA2), the latter a marker of smooth muscle cells.

D. As in A, except for co-staining with antibodies against TMEM43 and PECAM1,the latter a marker of endothelial cells.

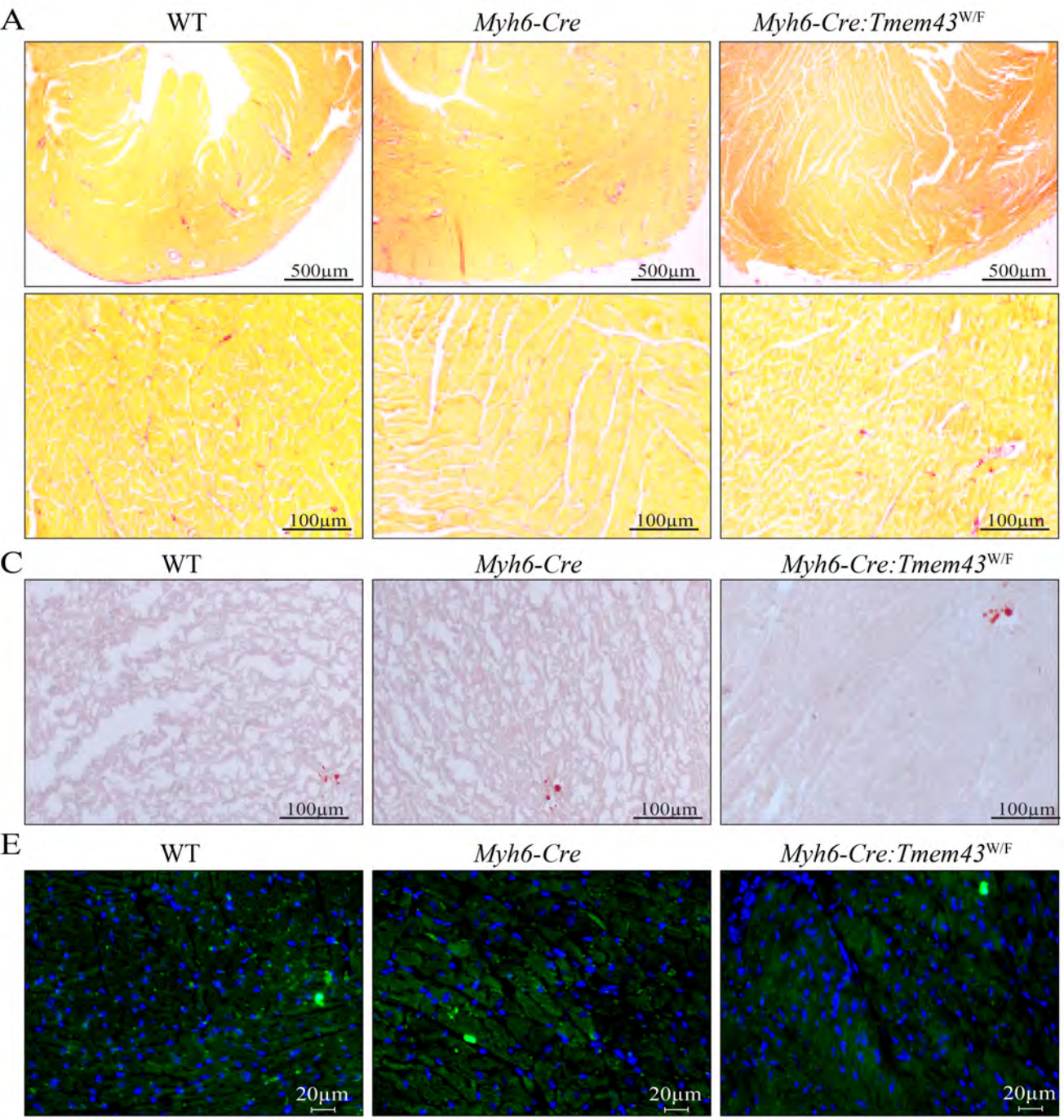
E. Immunoblots showing expression of TMEM43 protein in selected cardiac cell types

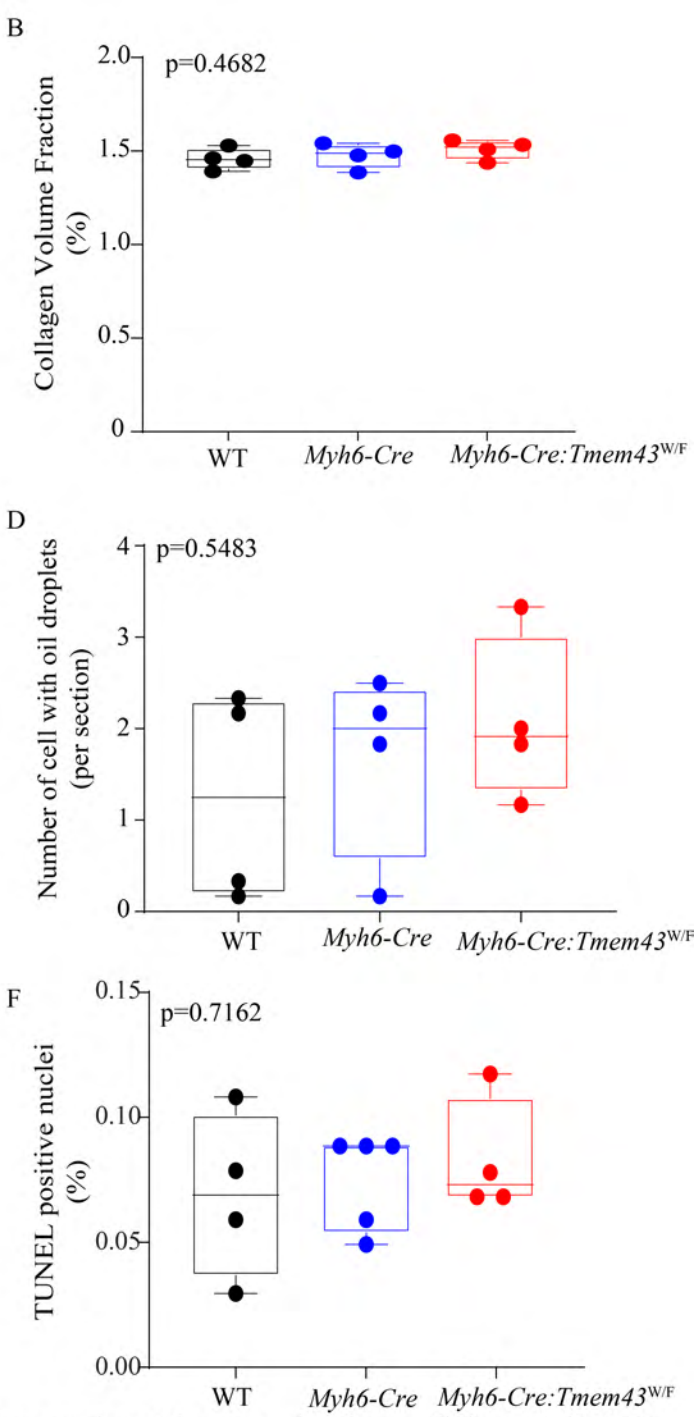


Online Figure 3. Recombination efficiency in the *Myh6-Cre:Tmem43*$^{W/F}$ mice.
A. Agarose gel electrophoregram showing PCR products of isolated cardiac myocyte genomic DNA in the wild type (WT), *Tmem43*$^{W/F}$ and *Myh6-Cre:Tmem43*$^{W/F}$ mice, which were used to calculate recombination efficiency. The floxed, WT, and the recombined alleles are identified by their size differences.
B. The stacking graph showing percent Cre-mediated recombined *Tmem43* allele in cardiac myocytes isolated from the *Myh6-Cre:Tmem43*$^{W/F}$ mice along with the controls.



Online Figure 4. TMEM43 protein levels in the Myh6-Cre mice.
Panel A. Immunoblots showing TMEM43 protein levels in cardiac myocytes isolated from the wild type and Myh6-Cre mice.
Panel B. Quantiative data representing the blots shown in Panel A





Online Figure 5. Myocardial histology in 4 months old *Myh6-Cre:Tmem43*$^{W/F}$ mice.

A and **B**. Panels showing picrosirius stained thin myocardial sections (**A**) along with the quantitative data (**B**) in the experimental groups, showing no differences in CVF among the groups (N=4).

C and **D**. Oil-Red O stained thin myocardial sections (**C**) along with the quantitative data (**D**) in the WT, *Myh6-Cre* and *Myh6:Cre-Tmem43*$^{W/F}$ mice.

E and **F**. Immunofluorescence images of TUNEL assay in the experimental groups (**E**) along with the quantitative data, showing no differences (**F**).

ANOVA p values are shown for each graph.

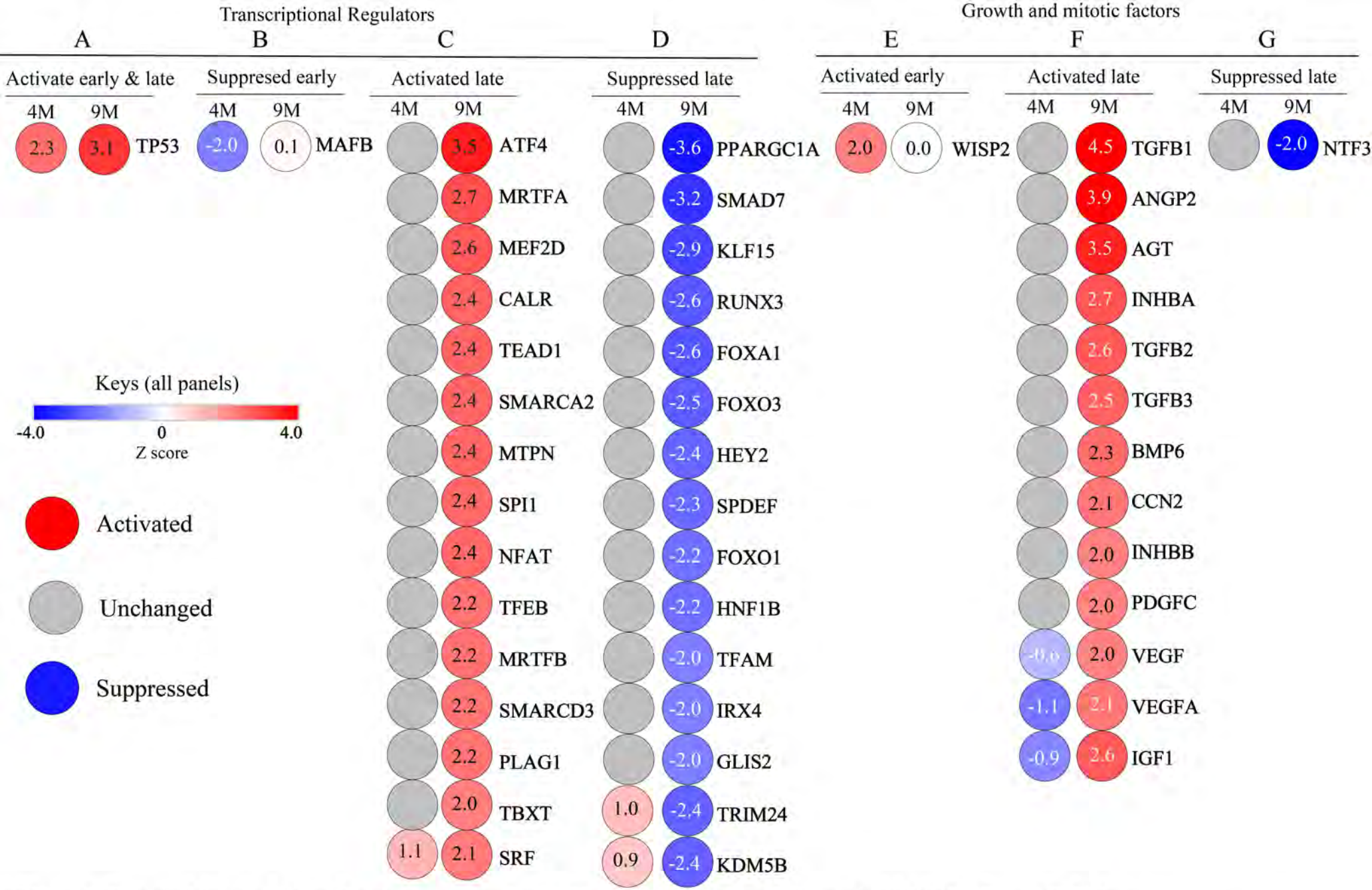


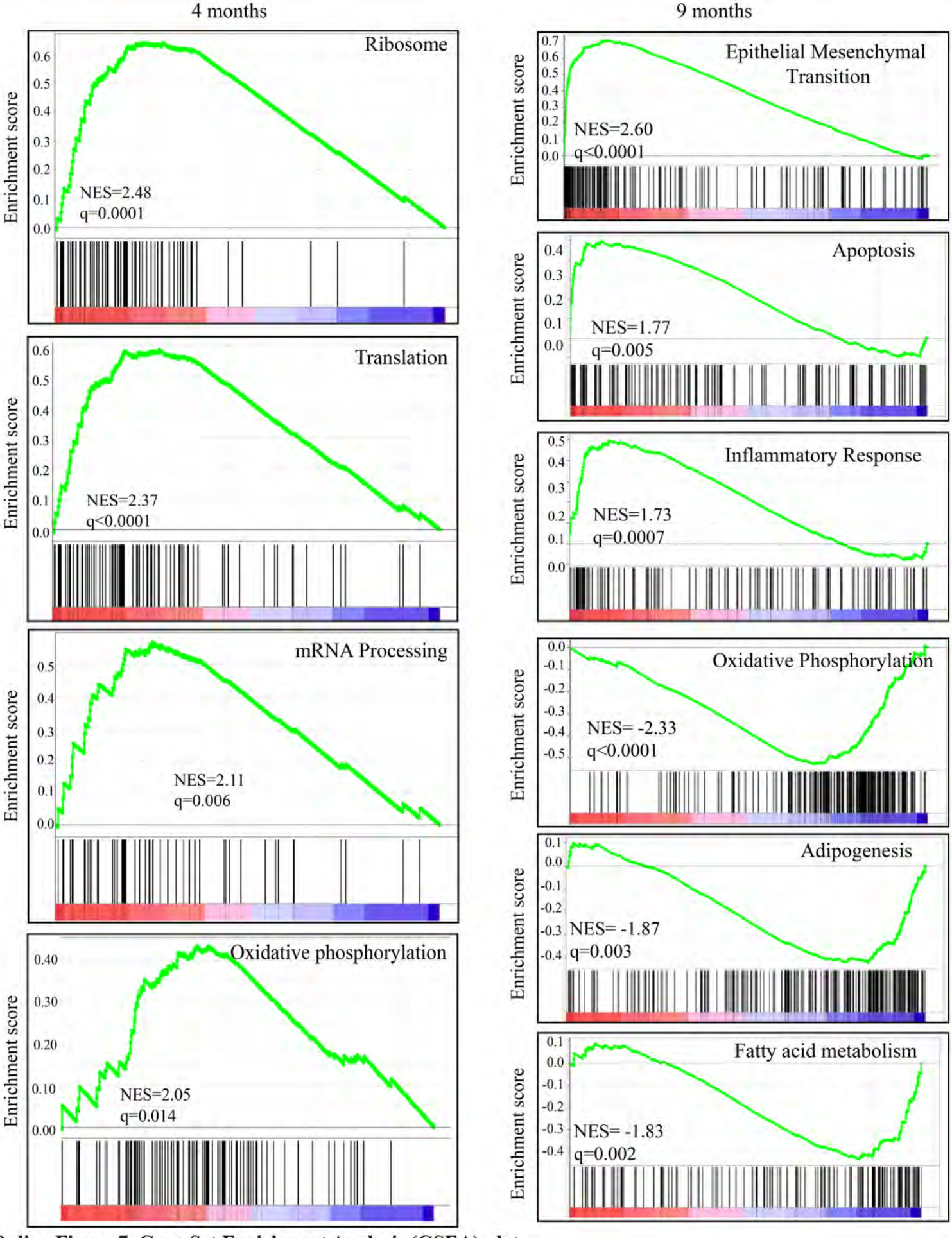
Figure 6. Temporal dysregulation of upstream transcriptional regulators and growth factors in *Myh6-Cre:Tmem43*$^{W/F}$ mice.

A. Only the TP53 was predicted to be activated at 4 months old *Myh6-Cre:Tmem43*W/F myocytes (Z score: 2.3), suggesting early activation of this pathway. The TP53 targets were further enriched and predicted enhanced activation at 9 months old *Myh6-Cre:Tmem43*W/F myocytes (Z score: 3.1).

B: DEGs predicted modest suppression of MAFB in cardiac myocytes from 4 months old *Myh6-Cre:Tmem43*W/F mice.

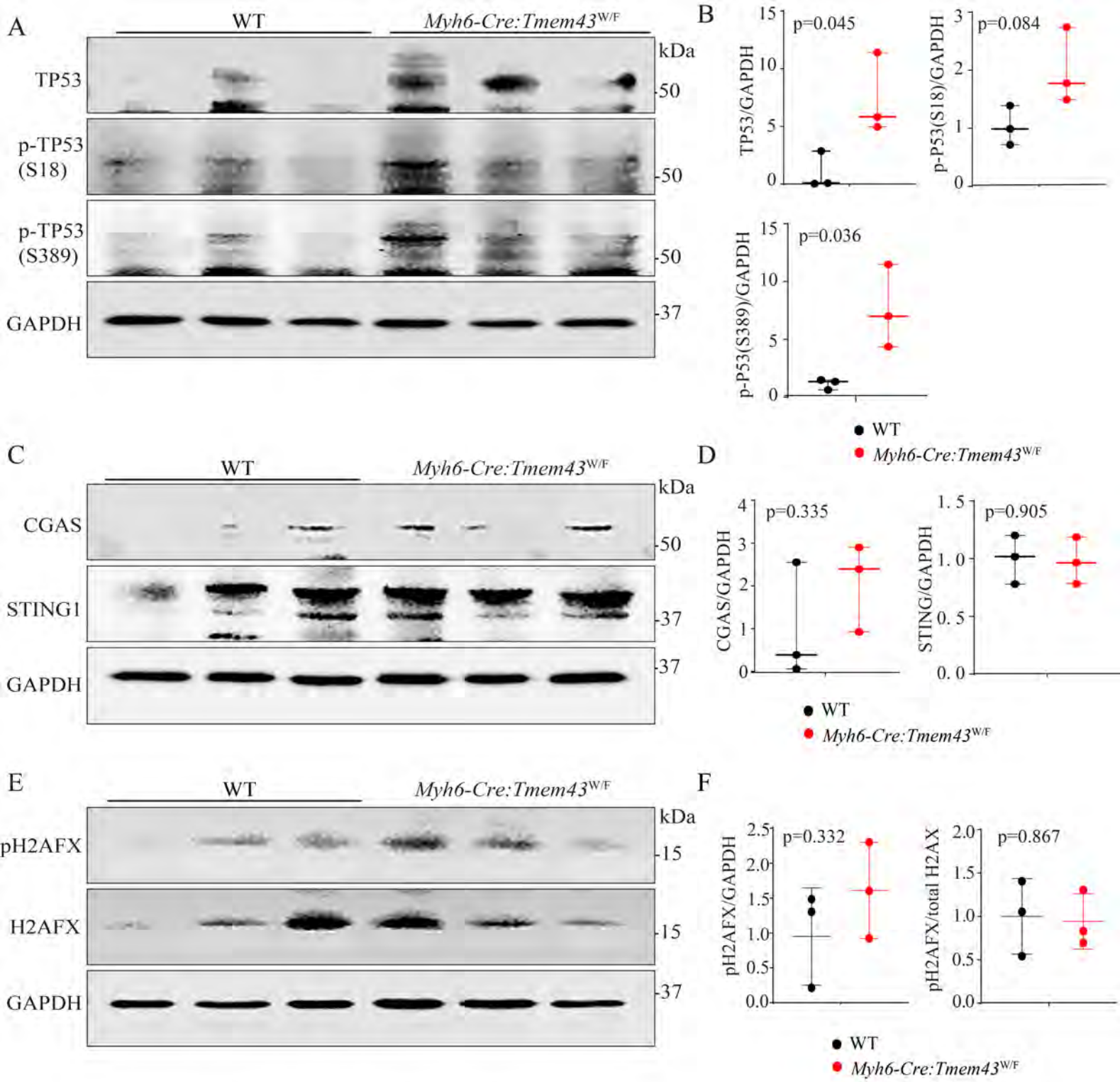
C and D. Enrichment of targets of over two dozen TRs in the DEGs predicting their activation (red), including TP53 (shown in panel A), ATF4, and MRTFA, whereas targets several TRs, such as PPARGC1A, SMAD7, and KLF15 were depleted (blue) in the 9 months old *Myh6-Cre:Tmem43*W/F myocytes.

E-G. Similar analysis to that in panels A-C suggesting activation (red) or suppression (blue) of growth and mitotic factors in the *Myh6-Cre:Tmem43*W/F cardiac myocytes at two time points. Notably, the DEGs predicted activation of TGFβ1 as the top growth and mitotic factor in the *Myh6-Cre:Tmem43*W/F myocytes



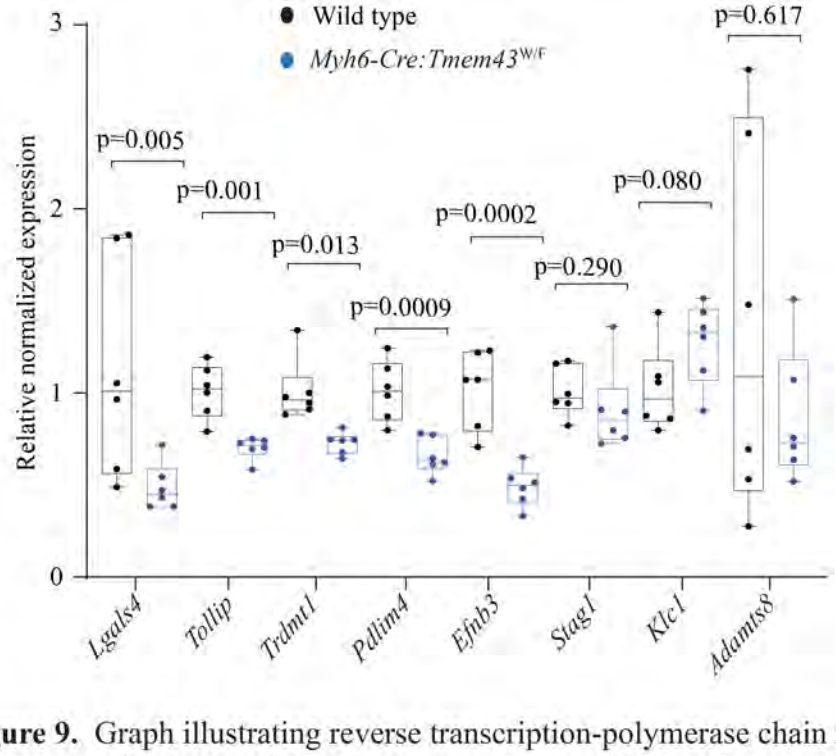
Online Figure 7. Gene Set Enrichment Analysis (GSEA) plots

GSEA plots for hallmark signatures obtained from RNA-seq data from ~ 4 and 9 months old *Myh6:Cre-Tmem43*$^{W/F}$ vs. WT comparisons. The plots depict the running Enrichment score (green line) and its correlation to the ranked gene list (black bar) in the *Myh6:Cre-Tmem43*$^{W/F}$ or WT group. Normalized enrichment score and q values are shown.

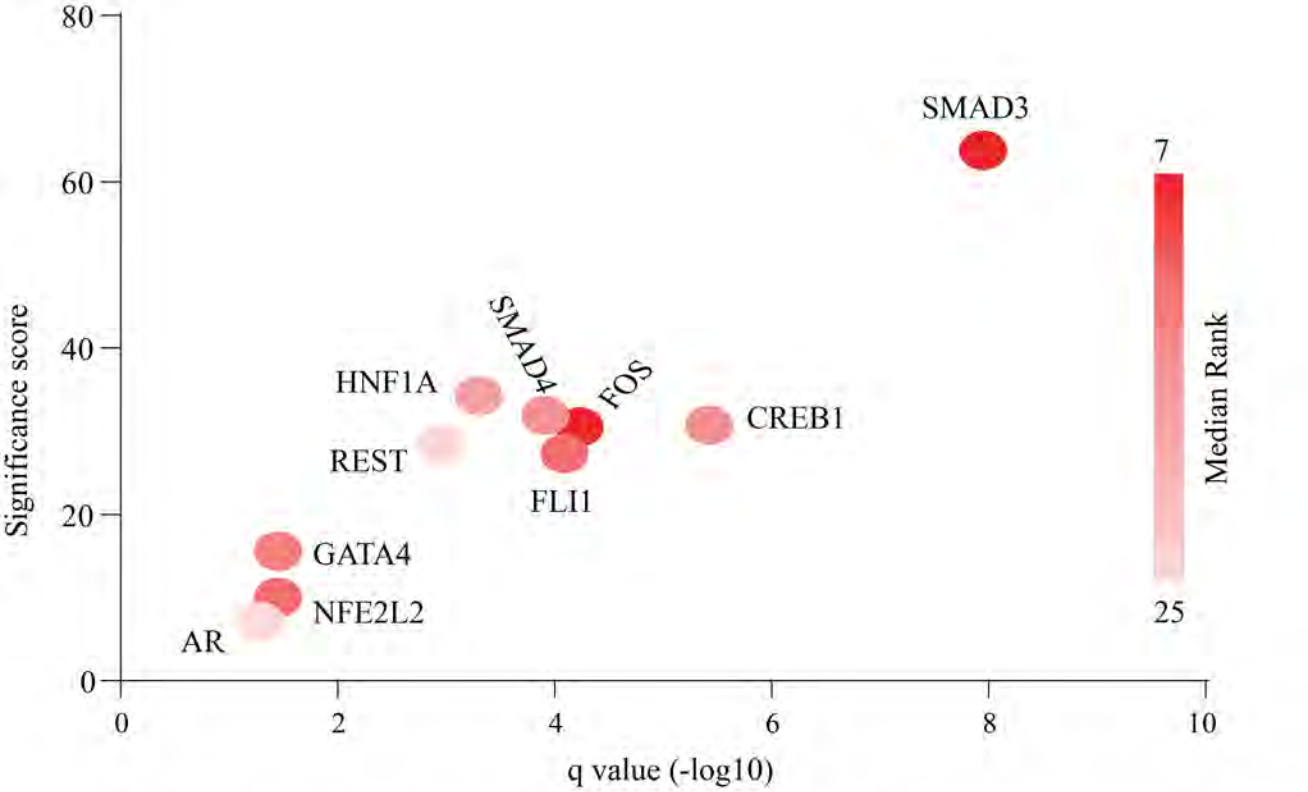


Online Figure 8. Immunoblot of selected protein in the DDR/TP53 pathway in the heart of 4 months old *Myh6-Cre:Tmem43*$^{W/F}$ mice

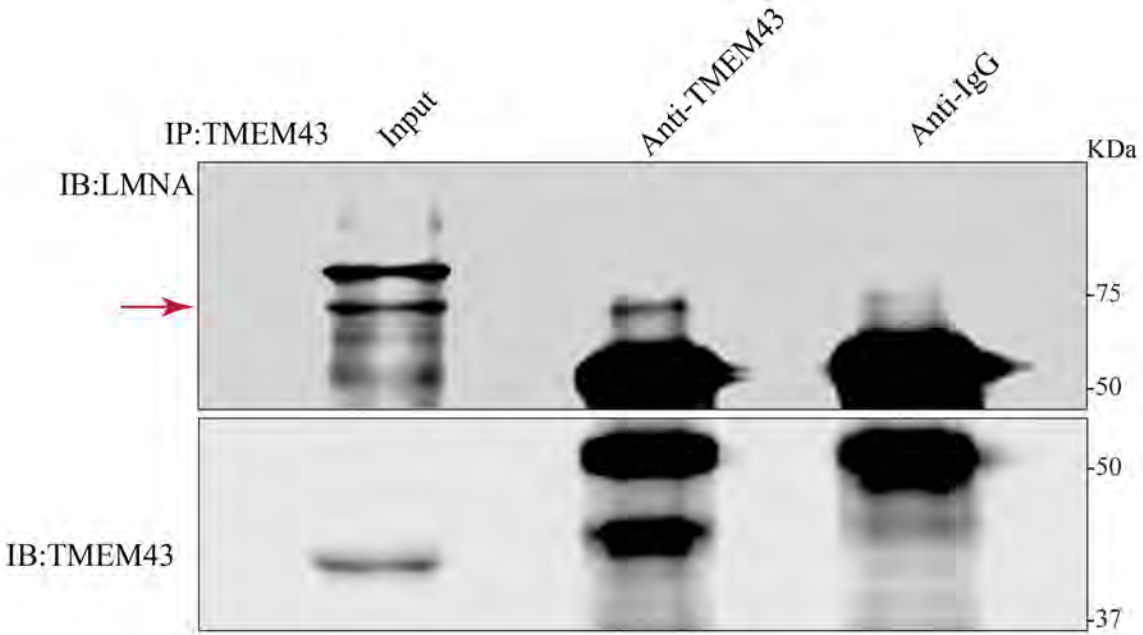
A. Immunoblots showing protein levels of total TP53 and phospho-TP53 (Serine 18 and Serine 389)
B. Quantitative data correspondng to blots in panel A, N=3 per group, p by unpaired t test
C. Immunoblots showing CGAS and STING1 protens of the DDR pathway
D. Quantitative data correspondng to blots in panel C, N=3 per group, p by unpaired t test
E. Immunoblots showing phospho-H2AFX and total H2AFX
F. Quantitative data corresponding to blots in panel E, N=3 per group, p by unpaired t test



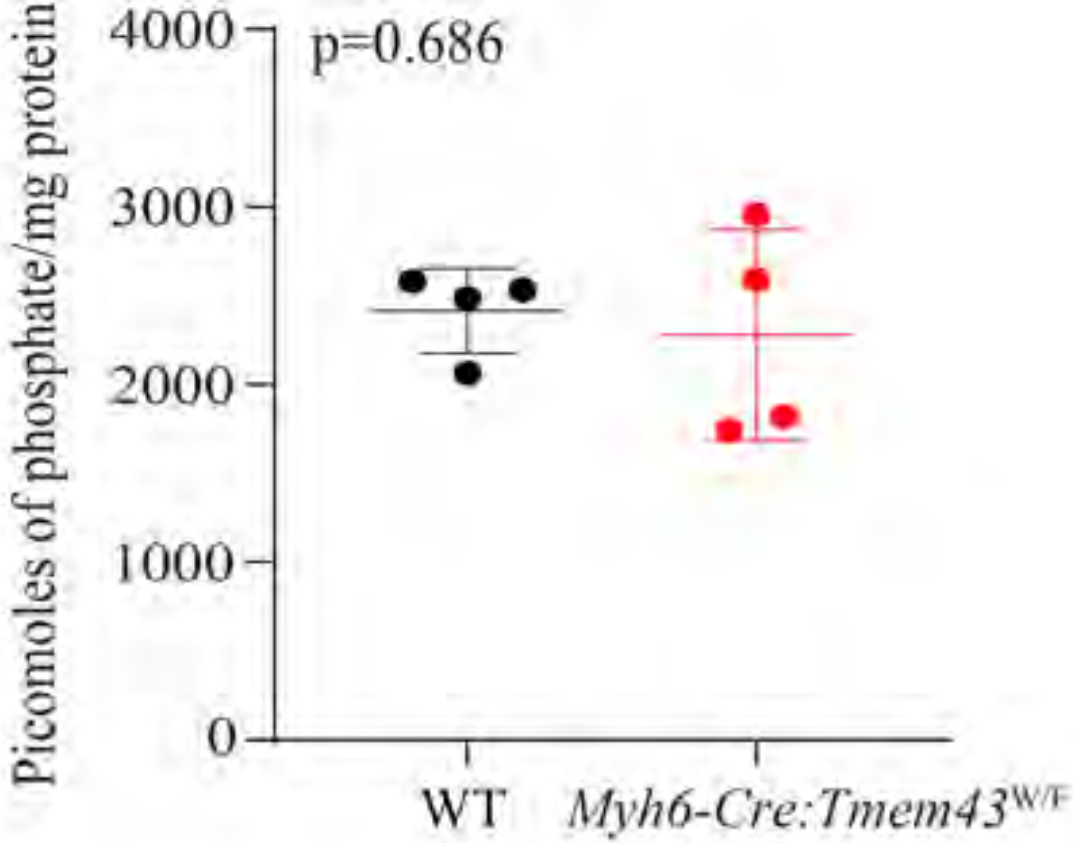
Online Figure 9. Graph illustrating reverse transcription-polymerase chain reaction (RT-PCR) data showing transcript levels of selected sensecence associated secretary phenotype (SASP) known to be downregulated upon activation of pathway in response to DNA damage (those increased are shown in the main figure 6).



Online Figure 10: Predicted dysregulated transcriptional regulators (TRs) and trophic and mitotic factors EGSEA analysis predicted dysregulated TRs based on the DEGs, which shows activation of SMAD3, the intracellular signal transducer of activated TGFβ1 pathway, as the top most activated TRs.



Online Figure 11. Co-immunoprecipitation of TMEM43 and Lamin A/C in adult mouse heart. Adult heart protein extracts were immunoprecipitated with an antibody against TMEM43 and immunoblotted and probed with an antibody against Lamin A/C (LMNA) protein. As shown in the upper panel, LMNA protein was immunoprecipitated with the TMEM43 antibody (arrow). Same blot was stripped and probed for TMEM43 to show the pull-down efficiency (lower panel).



Online Figure 12. PP2A activity in cardiac myocyte protein extracts
PP2A activity was measured using a commercially available kit in cardiac myocytes isolated from the 9 months old WT and *Myh6-Cre:Tmem43*$^{W/F}$ mice. N=4. p value was determined by unpaired student t-test.